Cortical substrate of bladder control in SCI and the effect of peripheral pudendal stimulation

Zempleni, M Z ; Michels, Lars ; Mehnert, U ; Schurch, B ; Kollias, S

Abstract: We investigate (i) the central representation of lower urinary tract (LUT) control and (ii–iii) the acute and 23 short-term central neuromodulatory effect of peripheral pudendal nerve stimulation in incomplete spinal 24 cord injured (SCI) patients using functional magnetic resonance imaging (fMRI). The urinary bladder of eight 25 SCI patients has been passively filled and emptied using a catheter, to identify the neural substrate of bladder 26 control (i), and with simultaneous peripheral pudendal nerve stimulation to investigate its acute central 27 neuromodulatory effect (ii). To identify the potential effects of pudendal nerve stimulation treatment (iii), 28 six patients underwent a 2-week training using pudendal nerve stimulation followed by another fMRI 29 session of bladder filling. The pre- and post-training fMRI results have been compared and correlated with 30 the patient's pre- and post-training urological status. Our results suggest that the central representation of 31 bladder filling sensation is preserved in the subacute stage of incomplete SCI. However, compared to earlier 32 data from healthy subjects, it shows decreased neural response in right prefrontal areas and increased in left 33 prefrontal regions, indicating diminished inhibitory micturition control as well as, compensatory or de- 34 compensatory reorganization of bladder control. We also provide evidence for a neuromodulatory effect of 35 acute pudendal nerve stimulation, which was most prominent in the right posterior insula, a brain region 36 implicated in homeostatic interoception in human. Pudendal stimulation training also induced significant 37 neuromodulation, predominantly signal increases, in the normal cortical network of bladder control. 38 Correlations with the patient's urological status indicate that this neuromodulatory effect may reflect the 39 clinical improvement following training.

DOI: https://doi.org/10.1016/j.neuroimage.2009.10.064

CORTICAL SUBSTRATE OF BLADDER CONTROL IN SCI AND THE EFFECT OF PERIPHERAL PUDENDAL STIMULATION

Monika-Zita Zempleni[1], Lars Michels[1,3], Ulrich Mehnert[2], Brigitte Schurch[2], Spyros Kollias[1]

1 Institute of Neuroradiology, University Hospital Zürich, Zürich, Switzerland

2 Neuro-Urology, Spinal Cord Injury Center, University of Zürich, Balgrist University Hospital, Zürich, Switzerland

3 Department of Functional Neurosurgery, University Hospital Zürich, Zürich, Switzerland

Neuroimage. 2010 Feb 15;49(4):2983-94

PMID: 19878725

DOI: 10.1016/j.neuroimage.2009.10.064

ABSTRACT

We investigated (i) the central representation of lower urinary tract (LUT) control and (ii–iii) the acute and short-term central neuromodulatory effect of peripheral pudendal nerve stimulation in incomplete spinal cord injured patients using functional magnetic resonance imaging (fMRI). The urinary bladder of eight SCI patients has been passively filled and emptied using a catheter, to identify the neural substrate of bladder control (i), and with simultaneous peripheral pudendal nerve stimulation to investigate its acute central neuromodulatory effect (ii). To identify the potential effects of pudendal nerve stimulation treatment (iii), six patients underwent a 2-week training using pudendal nerve stimulation followed by another fMRI session of bladder filling. The pre- and post-training fMRI results have been compared and correlated with the patient's pre- and post-training urological status. Our results suggest that the central representation of bladder filling sensation is preserved in the subacute stage of incomplete SCI. However, compared to earlier data from healthy subjects, it shows decreased neural response in right prefrontal areas and increased in left prefrontal regions, indicating diminished inhibitory micturition control as well as, compensatory or decompensatory reorganization of bladder control. We also provide evidence for a neuromodulatory effect of acute pudendal nerve stimulation, which was most prominent in the right posterior insula, a brain region implicated in homeostatic interoception in human. Pudendal stimulation training also induced significant neuromodulation, predominantly signal increases, in the normal cortical network of bladder control. Correlations with the patient's urological status indicate that this neuromodulatory effect may reflect the clinical improvement following training.

Keywords: Lower urinary tract; Spinal cord injury; Functional MRI; Neuromodulation; Pudendal nerve stimulation

INTRODUCTION

Efficient LUT control requires precise synchronization of an extended neural network including spinal and supraspinal structures. Primate studies reveal that four supraspinal anatomical regions are mainly involved in the regulation of the basic micturition/continence cycle [1-4]. The M region, i.e. Barrington nucleus or pontine micturition center, localized in the medial part of the dorsolateral pontine tegmentum, facilitates the motoneurons of the bladder and at the same time inhibits the motoneurons of the urethral sphincter through inhibitory interneurons. In contrast, a ventrolateral area of the pontine tegmentum, i.e. L region, sends excitatory input to motoneurons of the pelvic floor and external urethral sphincter and so doing facilitates continence [3, 4]. The PAG of the midbrain plays a role in transmitting sensory information related to bladder filling, whereas the preoptic region of the hypothalamus is hypothesized to be involved in the initiation of micturition [3]. Relatively few neuroimaging studies have addressed this issue until recently; the available results suggest that the anatomical structures described above play identical roles in LUT control in human [2].

Additionally, neuroimaging studies focusing on human micturition regulation (for recent reviews, see[1, 2, 5]), imply the involvement of an extensive suprapontine neural network, which is not surprising given the learned, social [1] and philogenetically rooted affective [6] aspects of this function.

These studies revealed that bladder sensation, i.e. increasing levels of bladder filling, in healthy subjects is accompanied by BOLD changes in the anterior cingulate gyrus, lateral prefrontal cortex, insula (mostly in the right hemisphere), thalamus, hypothalamus, PAG, pons, bilateral occipito-parietal regions and in the cerebellum [7-13].

During successful micturition in the PET scanner [12, 13], increased activation has been reported in the right inferior frontal gyrus, right anterior cingulate, dorsal pons, left medial frontal gyrus, right medial temporal gyrus, hypothalamus and PAG. In the same studies, involuntary inhibition of micturition was associated with activations in the ventral pontine tegmentum, the right inferior frontal and the right anterior cingulate gyri. In a previous fMRI study [14], we identified brain regions involved in intentional micturition inhibition; these were bilateral medial frontal areas with clear right dominance, bilateral putamen, right parietal cortex, right limbic system and right cerebellum. These studies suggest that the right frontal lobe may be important for inhibiting voiding when the bladder is distended but micturition is not possible; e.g. socially inacceptable.

Identifying the cortical substrate of LUT control, especially in patient populations, may facilitate efficient treatments for common bladder dysfunctions, such as overactive bladder, which can be either idiopathic [8, 15] or subsequent to various neurological disorders. For example, the bladder in SCI patients becomes hyperreflexic followed by the development of negative detrusor-sphincter synergy, i.e. the simultaneous occurrence of irregular detrusor and sphincter contractions. Eventually, this leads to involuntary micturitions, incomplete bladder emptying, increased risk for bladder infections and high urine pressures damaging the upper urinary tracts. It is hypothesized that detrusorsphincter dyssynergia in SCI is caused by injured ponto-spinal, as well as from emerging pathological peripheral reflexes (see e.g.[16, 17]). It is not clear whether suprapontine neural changes also contribute to, or at least follow, the pathological LUT function in SCI patients.

Bladder dysfunction impairs life quality in SCI patients to a great extent, as most of them require permanent or intermittent catheterization [18, 19] and antimuscarinic medication. Electrical pudendal nerve stimulation emerged as a promising therapeutic approach for improving LUT function in conditions such as sphincter weakness or overactive bladder [20-23] despite controversies regarding its efficacy and side-effects [24-29]. The mechanism of its therapeutic effect is also yet to be clarified [21].

In the current study, we investigate whether SCI alters the central neural pattern of LUT control by comparing the cortical substrate of bladder filling sensation in incomplete SCI patients with those of healthy subjects using fMRI. Our hypothesis was that the SCI condition would alter the central representation of LUT control reflecting the functional disabilities of these patients. In the same patient population, we further evaluated the acute and short-term effect of peripheral pudendal nerve stimulation on the cortical substrate of LUT control. Our hypothesis was that pudendal stimulation has a central neuromodulatory effect, additionally to a sole peripheral one, that would be revealed by changes in the fMRI pattern both during the acute and the short-term stimulation settings. Based on earlier clinical reports, we expected that pudendal nerve stimulation will improve LUT function of the incomplete SCI patients and this clinical change would be accompanied by a “normalization” of the neural activation pattern; i.e. the neural pattern of LUT control after 2 weeks of pudendal stimulation treatment would be similar to that of healthy subjects.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL SETUP

The study has been approved by the local Ethics Committee (Kantonale Ethikkomission Zürich). Subjects signed written informed consent prior to participation in accordance with the Declaration of Helsinki. The study consisted of three parts: (1) 1st fMRI session, (2) followed by 2-week electrical pudendal nerve stimulation training and a (3) 2nd, follow-up fMRI session. During the 1st fMRI session, two experiments have been carried out; (i) a bladder filling paradigm has been performed to identify the central representation of urinary bladder filling sensation (hence referred to as *bladder filling experiment*), (ii) the same bladder filling paradigm has been repeated with simultaneous pudendal nerve stimulation to identify its potential acute neuromodulatory effects (hence *acute pudendal stimulation experiment*).

During the 2nd fMRI session, the bladder filling paradigm has been repeated; these data were then compared with the bladder filling data from the 1st fMRI session to reveal potential neuromodulatory effects of the intervening short-term, i.e. 2-week, peripheral pudendal stimulation training (hence *short-term pudendal stimulation experiment*).

BLADDER FILLING PARADIGM

Patients have been catheterized prior to entering the MRI scanner; i.e. they had either a permanent suprapubic catheter or were catheterized using a standard transurethral Foley catheter (see **Table 1**). After positioning the subjects in the scanner, their urinary bladder has been pre-filled with sterile saline solution until the 'first desire to void' occurred. In those patients who did not have this sensation, 200 ml volume has been used for pre-filling. During the experiment, the bladder has been repeatedly filled and emptied (80 ml sterile saline) via the catheter. The paradigm was a block design in which four conditions followed each other: rest (30 s) – bladder filling (15 s) – rest with full bladder (30 s) – bladder emptying (15 s). This cycle was repeated five times.

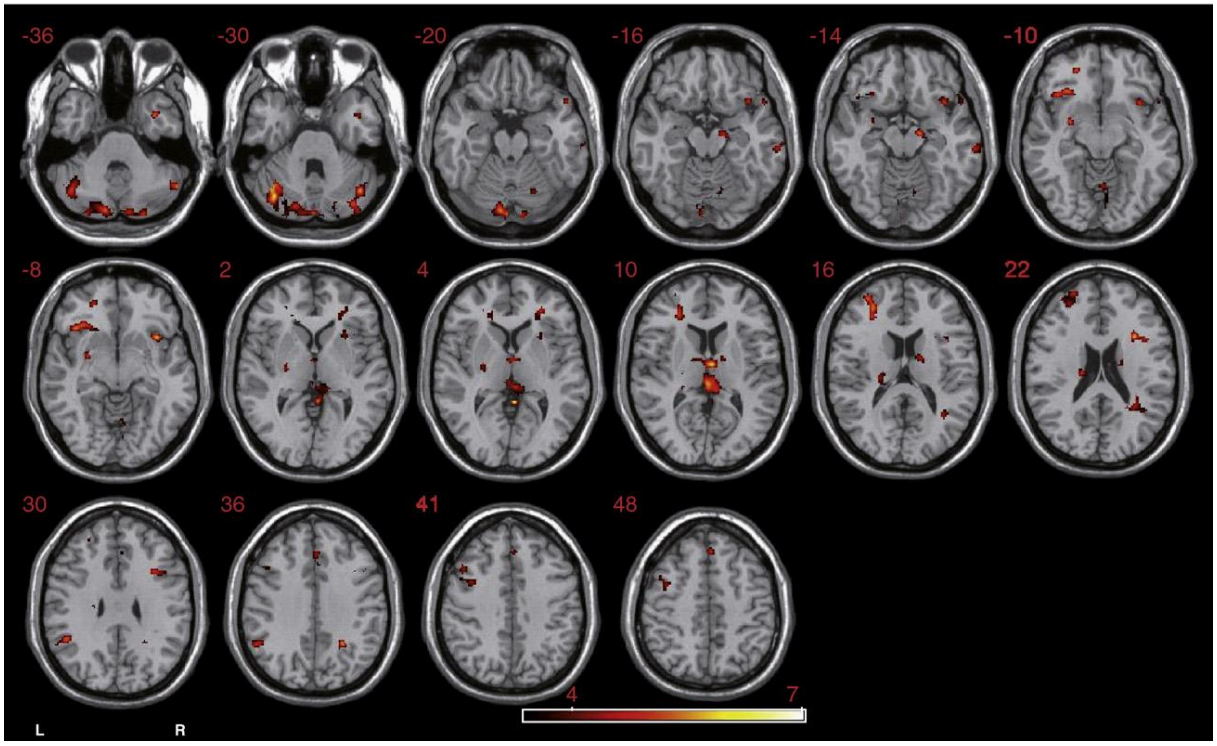

Figure 1 The neural substrate of bladder filling sensation. Bladder filling>rest contrast. Data from the 1st fMRI session, before treatment, eight subjects. Areas were significant at a voxel threshold of p (FDR correction)≤0.01 and cluster extent threshold≥50 voxels (corresponding to T≥3.43 and p voxel level uncorrected≤0.001). The MNI normalized T-map is overlaid on a standard MNI template using MRIcro (Rorden and Brett, 2000).Numbers indicate the MNI z coordinates of the slices; the slices are displayed in neurological convention, i.e. left side in the figure corresponds to the left hemisphere. All slices at which an activation maximum has been found are shown, unless two slices would show the same clusters at different heights.

IMAGE ACQUISITION

MRI data acquisition took place at the MR Center of the University Hospital of Zurich using a 3-Tesla MR scanner with SENSE head coil. BOLD sensitive, single-shot, gradient echo, EPI sequences (TE: 35 ms, TR: 3000 ms, Flip angle: 82°, FOV: 220 × 220 mm, matrix: 128 × 128, slice thickness: 3 mm, 39 slices covering the entire brain, except the most caudal part of the cerebellum) were acquired. One hundred fifty whole brain data have been collected during each experiment.

PUDENDAL NERVE STIMULATION

The *2-week pudendal stimulation training* took place in the Neuro- Urology Department of the Spinal Cord Injury Center (University of Zurich, Balgrist University Hospital, Zurich). The peripheral pudendal nerve was stimulated for 15 min twice daily using two, self-adhesive disc electrodes symmetrically attached to the penis/clitoris of the participants; i.e. the dorsal penile/clitoral branches of the pudendal nerve have been stimulated. The stimulation device produced symmetric, biphasic, square-wave impulses with fixed pulse width of 0.2 ms, frequency of 20 Hz and non-painful intensity range of 10–80 mA. These parameters have been found to be efficient in earlier studies (for review see [21]).

In the scanner during the *acute pudendal stimulation experiment*, a custom made MR compatible pneumatic device (for details see [30]) has been used to elicit tactile stimulation of the pudendal nerve. The device releases boluses from a compressed-air bottle with the help of an electromagnetic valve and a trigger box, which are transmitted to the penile/clitoral skin of the subjects via plastic tubes and membranes, thus producing efficient peripheral pudendal nerve stimulation in the scanner.

SUBJECTS

Eight right handed subjects (4 females and 4 males) participated in the 1st fMRI session (i.e. bladder filling and acute pudendal stimulation experiments). Six of them (3f and 3m) have also completed the 2-week stimulation treatment and the 2nd fMRI session (i.e. short-term pudendal stimulation experiment). They were paraplegic inpatients, recruited from the wards of the Spinal Cord Injury Center (University of Zurich, Balgrist University Hospital, Zurich).

All but one patient had incomplete SCI according to the American Spinal Cord Injury (ASIA) classification system, i.e. ASIA B or C. We also included one complete (ASIA A) subject, since he had some degree of maintained bladder sensation. This exception has been made on the basis that the ASIA classification system focuses on sensory and motor functions, whereas in the current study we gave priority to bladder status and bladder sensation. Seven patients suffered from detrusor overactivity and one subject had flaccid bladder. Patients with systematic neurological disorders or any kind of brain pathology, which might have altered the fMRI pattern, have not been included. Participants' demographic and clinical data (before and after the pudendal nerve stimulation training) are reported in **Table 1**.

DATA ANALYSIS

The fMRI data were analyzed using Statistical Parametric Mapping SPM2). First spatial preprocessing took place including realignment and MNI normalization. Smoothing followed using a 6 mm Gaussian kernel; relatively small kernel width was chosen since regions of interests included small anatomical areas, e.g. PAG in the midbrain. The preprocessed images were used for the statistical analysis as described below.

(i) In order to evaluate the *neural substrate of bladder filling sensation*, a fixed-effects group analysis has been carried out. The images from the bladder filling experiment of the eight participants from the 1st fMRI session have been entered into one design matrix; statistical values have been estimated using the general linear model, then the bladder filling condition was contrasted with the empty bladder condition. For using equal condition lengths and avoiding potential confounding effects from the bladder emptying condition, we only used the second half of the empty bladder condition for the statistical comparison. Therefore, 15 s of bladder filling were contrasted with an equally long period of empty bladder at rest; hence referred to as bladder filling>rest contrast (see **Figure 1** under the Results section and **Supplementary Table A**).

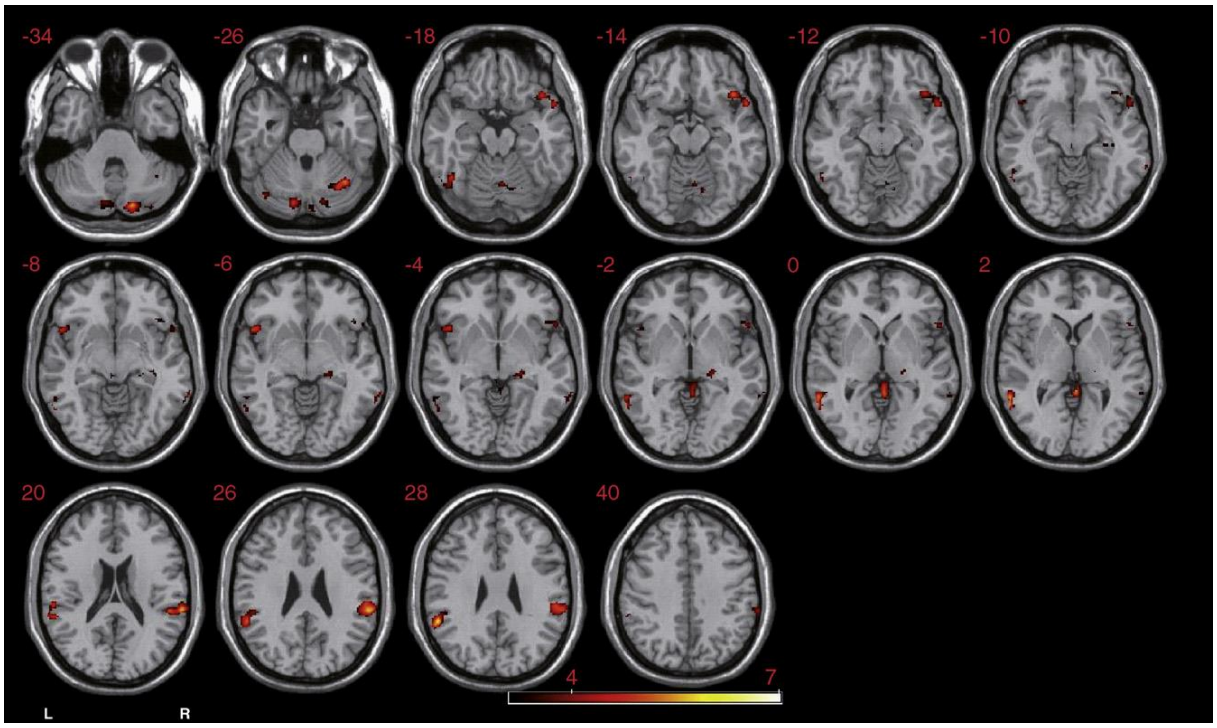

Figure 2 Acute effect of simultaneous pudendal nerve stimulation on the neural substrate of bladder filling sensation. Bladder filling with simultaneous pudendal nerve stimulation>rest contrast. Data from the 1st fMRI session, eight subjects. Areas were significant at a voxel threshold of p (FDR correction)≤0.01 and cluster extent threshold≥50 voxels (corresponding to T≥3.84 and p voxel level uncorrected≤0.001). The MNI normalized T-map is overlaid on a standard MNI template using MRIcro (Rorden and Brett, 2000). Numbers indicate the MNI z coordinates of the slices; slices are displayed in neurological convention, i.e. left side in the figure corresponds to the left hemisphere. All slices at which an activation maximum has been found are shown, unless two slices would show the same clusters at different heights.

For identifying the anatomical regions, we used multiple resources, such as the Automated Anatomical Labeling toolbox from SPM2 [31] and the Talairach and Tournoux [38] Co-Planar Stereotaxic Atlas; for the latter, the MNI coordinates have been converted into Talairach and Tournoux coordinates using two conversion algorithms (mni2tal and icbm_spm2tal; see e.g. [32] and [33]). Priority was given to anatomically identifying the activated regions by overlying the MNI normalized statistical maps on a standard MNI template using MRIcro [34]. The same procedure was used to evaluate the other contrasts as well (see below).

(ii) To evaluate the *acute neuromodulatory effect of pudendal nerve stimulation*, the images from the acute pudendal stimulation experiment, i.e. data from eight subjects from the 1st fMRI session, have been analyzed identically as described above under (i). The results of the relevant contrast from this analysis, i.e. bladder filling with simultaneous pudendal nerve stimulation>rest contrast, are reported in **Figure 2** under the Results section and **Supplementary Table B**.

Table 1 Demographic(a, b), clinical(c–h) and cystometric(i–q) data of participants before (A) and after (B) pudendal nerve stimulation.

Age[a]	Sex[b]	ASIA[c]	Level[d]	E[e]	Time[f]	DO[g]	Voiding[b]	Urodynamical data								
								IWT[i]	FSF[j]	FDV[k]	SDV[l]	MCC[m]	pDET max[n]	BC[o]	VV[p]	PVRV[q]
A																
p1 59	F	C	C5	T	1	Yes	SPC	+ (63)	-	-	-	425*	22	30	-	-
p2 23	M	B	Th8	T	2.3	Yes	SPC	- (26)	-	-	-	330	35	24	-	-
p3 72	F	C	C5	T	2.8	Yes	CISC	+ (30)	-	-	-	500	20	22	-	-
p4 30	M	C	C6	T	0.8	Yes	voluntary (PVRV: 250 ml)	+ (55)	260	350	500	500	29	21	-	-
p5 44	F	C	Th11	I	52	No	CISC	- (20)	-	-	-	500	10	> 100	-	-
p6 54	M	A	Th5	T	2.6	Yes	CISC	+ (40)	300	-	-	500	18	50	-	-
p7 70	F	B	C6	I	1.7	Yes	Foley	+ (54)	-	-	-	426	63	10	-	-
p8 19	M	B	Th7	T	2.5	Yes	SPC	+ (76)	-	-	-	180	64	16	-	-
B																
p1 59	F	C	C5	T	1	Yes	SPC	-**	254	326	361	400	28	> 100	50	350
p2 23	M	B	Th8	T	2.3	No	CISC	-	225	240	300	300	10	21	150	150
p3 72	F	C	C5	T	2.8	No	CISC	-	248	661	950	950	14	46	450	500
p4 30	M	C	C6	T	0.8	Yes	Voluntary	-	None	330	None	330	38	165	237	93

(A) The first six subjects (Pat. p1–p6) participated in both scanning sessions; the last two subjects only in the 1st fMRI session. Age(a): age of patients in years. Sex(b): female (F) and male (M). ASIA(c) A, B and C refers to the standard classification according to the American Spinal Injury Association. B and C indicate incomplete SCI whereas A indicates complete SCI. As can be seen all patients had incomplete SCI except one subject who was included based on the, to some extent, maintained bladder sensation. Level(d) of the SCI was either cervical (C) or thoracic (Th). Etiology (E(e)) of the SCI was either traumatic (T) or iatrogenic (I); iatrogenic SCI was caused by complicated epidural and periradicular injections. Time (f) indicates the duration of the SCI at the time of the 1st fMRI session expressed in months. The presence of detrusor overactivity (DO)(g) is indicated with "yes" or "no". Voluntary voiding(h) was only possible in one subject (p4), however, with a post-void residual volume (PVRV) of 250 ml. The other subjects required permanent catheterization by suprapubic catheter (SPC), Foley catheter or clean intermittent self-catheterization (CISC). Columns (i–o) show the results of the Cystometry which took place mostly before the1st fMRI session. The ice water test (IWT)(i) was either positive or negative; the numbers in brackets show pressure values given in cm H2O. As can be seen, most subjects had no first sensation of filling (FSF)(j), first desire to void (FDV)(k) or strong desire to void (SDV)(l). In case when these sensations were present, the volume at which the sensation has occurred is expressed in milliliters. Maximum cystometric capacity (MCC)(m) at which volume leakage occurred is expressed in milliliters. In one subject, marked with ☐, abdominal and leg spasms occurred at 425 ml infusion without leakage. In all subjects, maximum detrusor pressure (pDET max)(n) was determined and expressed in cm H2O. Column (o) indicates the degree of bladder compliance (BC), column (p) indicates voided volume (VV) and column (q) indicates post void residual volume (PRVR).
(B) Clinical and urodynamical data of the four subjects who underwent the 2-week electrical pudendal stimulation training and participated in both scanning sessions. These changes have been evaluated directly after the 2nd fMRI session. The subject numbers (p1–p4) correspond to subject numbers in (A). The urodynamically observed improvements corresponded to the subjective perceptions of the 4 patients, who indicated improved bladder sensation and improved voluntary voiding. ☐☐ indicates that the IWT was only performed before the pudendal stimulation training.

For quantifying the differences between the bladder filling with and without pudendal nerve stimulation conditions, we contrasted the bladder filling vs. bladder filling with simultaneous pudendal nerve stimulation conditions in both directions. In this analysis, a design matrix was built and estimated containing the bladder filling and the acute pudendal stimulation experiments from the eight subjects who took part in the 1st fMRI session; see **Supplementary Table C** and **Figure 3** under the Results section.

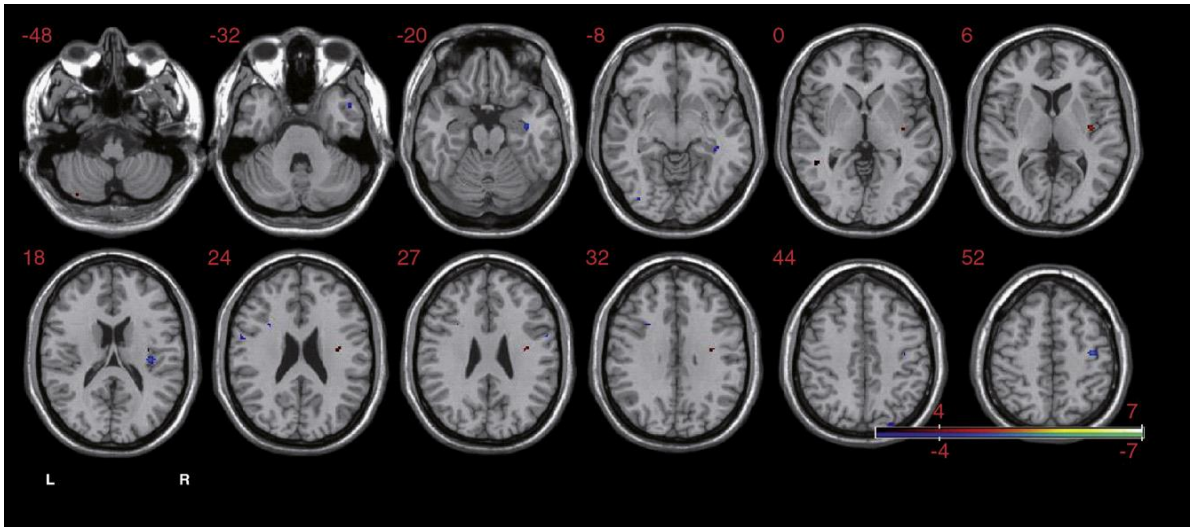

Figure 3 Acute effect of simultaneous pudendal nerve stimulation on the neural substrate of bladder filling sensation. Bladder filling vs. bladder filling with simultaneous pudendal nerve stimulation contrast; i.e. this figure is the statistical comparison of the activation patterns shown in **Figure 1** and **Figure 2**. Data from the 1st fMRI session, eight subjects. Areas are shown in which the simultaneous pudendal nerve stimulation elicited significant change in either direction at the voxel threshold of p uncorrected≤0.001 and cluster extent threshold≥10 voxels (corresponding to T≥3.1). Anatomical regions, which showed BOLD decrease due to the simultaneous pudendal stimulation, are shown in blue shades whereas areas, which showed BOLD increase, are presented in red shades. In the corresponding **Supplementary Table C**, the same 'color code' has been applied. The MNI normalized T-map is overlaid on a standard MNI template using MRIcro (Rorden and Brett, 2000). Numbers indicate the MNI z coordinates of the slices; the slices are displayed in neurological convention, i.e. left side in the figure corresponds to the left hemisphere. All slices at which an activation maximum has been found are shown, unless two slices would show the same clusters at different heights.

(iii) To investigate the *short-term neuromodulatory effect of the 2 weeks pudendal nerve stimulation treatment*, data from the bladder filling experiments from the 1st and the 2nd fMRI sessions have been used from those six participants who took part in both fMRI sessions. This way, an equal number of pre- and post-training fMRI runs, i.e. two times six runs, has been entered into one design matrix and fixed-effects group statistics were calculated. After estimation, the bladder filling conditions from the two scanning sessions, i.e. bladder filling prior vs. after pudendal stimulation treatment, have been contrasted in both directions (see **Figure 4** under the Results section and **Supplementary Table B** and **Supplementary Table D**).

The clinical LUT status of the six participants has been evaluated (**see Table 1B**) and a group analysis has been performed for the four participants showing change in their clinical LUT status (see **Figure 5** under the Results section and **Supplementary Table E**).

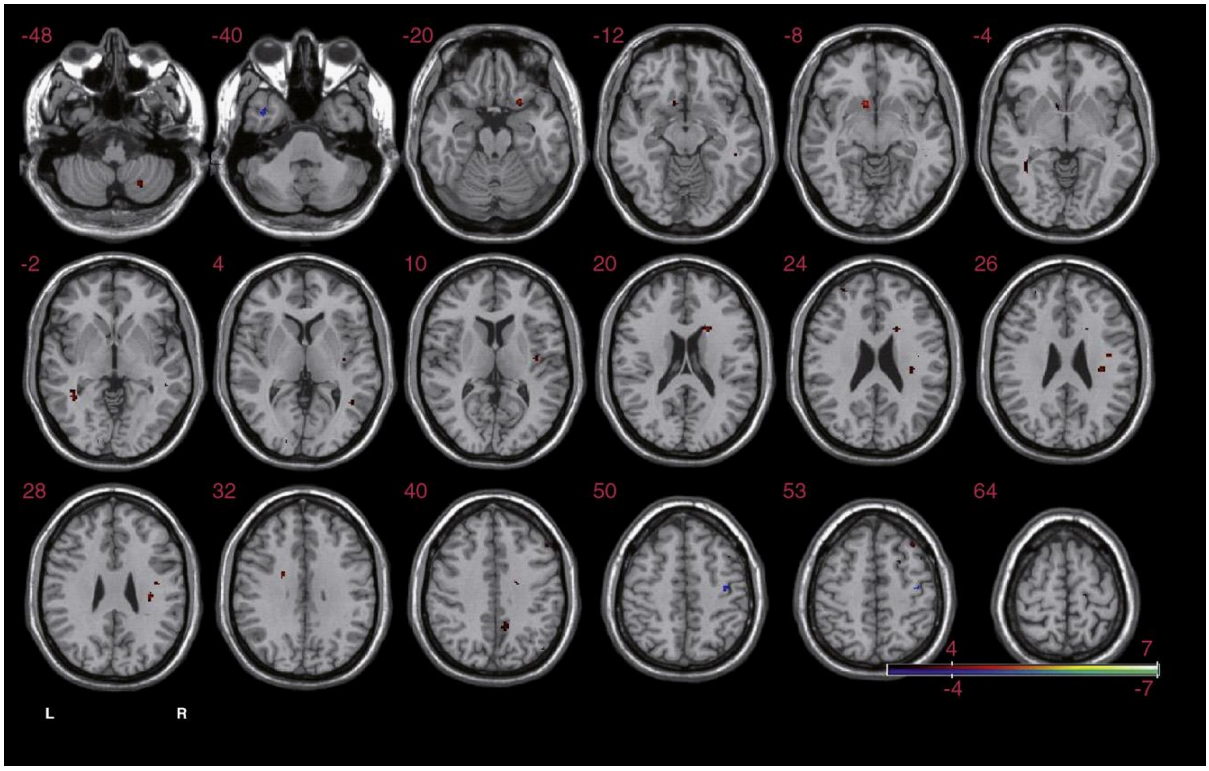

Figure 4 Effect of 2-week pudendal nerve stimulation in six subjects. Bladder filling prior vs. bladder filling following the 2-week pudendal nerve stimulation treatment evaluated in all six participants irrespective of their clinical response. Data from the 1st vs. 2nd fMRI sessions: areas are shown in which the 2-week pudendal nerve stimulation elicited significant change in either direction at the voxel threshold of p uncorrected≤0.001 and cluster extent threshold≥10 voxels corresponding to T≥3.1. Anatomical regions which showed BOLD decrease after treatment are shown in blue shades whereas areas in which the BOLD increased are presented in red shades. In the corresponding **Supplementary Table D**, the same 'color code' has been applied. The MNI normalized T-map is overlaid on a standard MNI template using MRIcro (Rorden and Brett, 2000). Numbers indicate the MNI z coordinates of the slices; the slices are displayed in neurological convention, i.e. left side in the figure corresponds to the left hemisphere. All slices at which an activation maximum has been found are shown, unless two slices would show the same clusters at different heights.

Finally, the effect of the 2-week pudendal stimulation treatment has also been evaluated on the individual level. In this analysis, the conditions from the bladder filling before treatment vs. bladder filling after treatment have been entered into one design matrix and the contrast was calculated for each individual participant (see **Figure 6** and **Figure 7** under the Results section).

(iv) The M and L regions of the pons, as well as PAG of the midbrain are consistently reported as parts of the neural substrate of LUT control (see Introduction). However, these are fairly small areas. Therefore, each contrast, described under (i), (ii) and (iii), has also been evaluated at a lower significance and extent threshold in these regions of interest.

RESULTS

THE NEURAL SUBSTRATE OF BLADDER FILLING SENSATION

The contrast bladder filling>rest from the 1st fMRI scanning session revealed significant activation of several supra- and infratentorial brain areas shown in **Figure 1**. This figure shows the results at a

voxel threshold of *p* (FDR correction)≤0.01 and a cluster extent threshold (kE)≥50 voxels; corresponding to T≥3.43 and *p* uncorrected≤0.001. The complete and detailed list of activated clusters is provided in **Supplementary Table A**.

The most prominent activation clusters were found in the cerebellar hemispheres bilaterally, with right dominance, and in the vermis. Extensive activations were also seen in the thalamus and putamen bilaterally, in the left caudate and in the left frontal lobe, especially the middle frontal gyrus (BA 10, 8 and 6) extending into the superior frontal (BA 9) and precentral (BA 6) gyri. Other significant activations were found in the insula bilaterally, extending into the inferior frontal gyri, the anterior cingulate, predominantly right hemispheric, the neighboring frontal gyri (BA 24, 32 and 8) as well as in the posterior cingulate (BA 31) and the right precuneus (BA 7). Smaller, but still significant, activations were seen in the left supramarginal gyrus (BA 40), right substantia nigra of the midbrain, right superior temporal gyrus (temporo-polar BA 38) and the right middle temporal gyrus (BA 20 and 21).

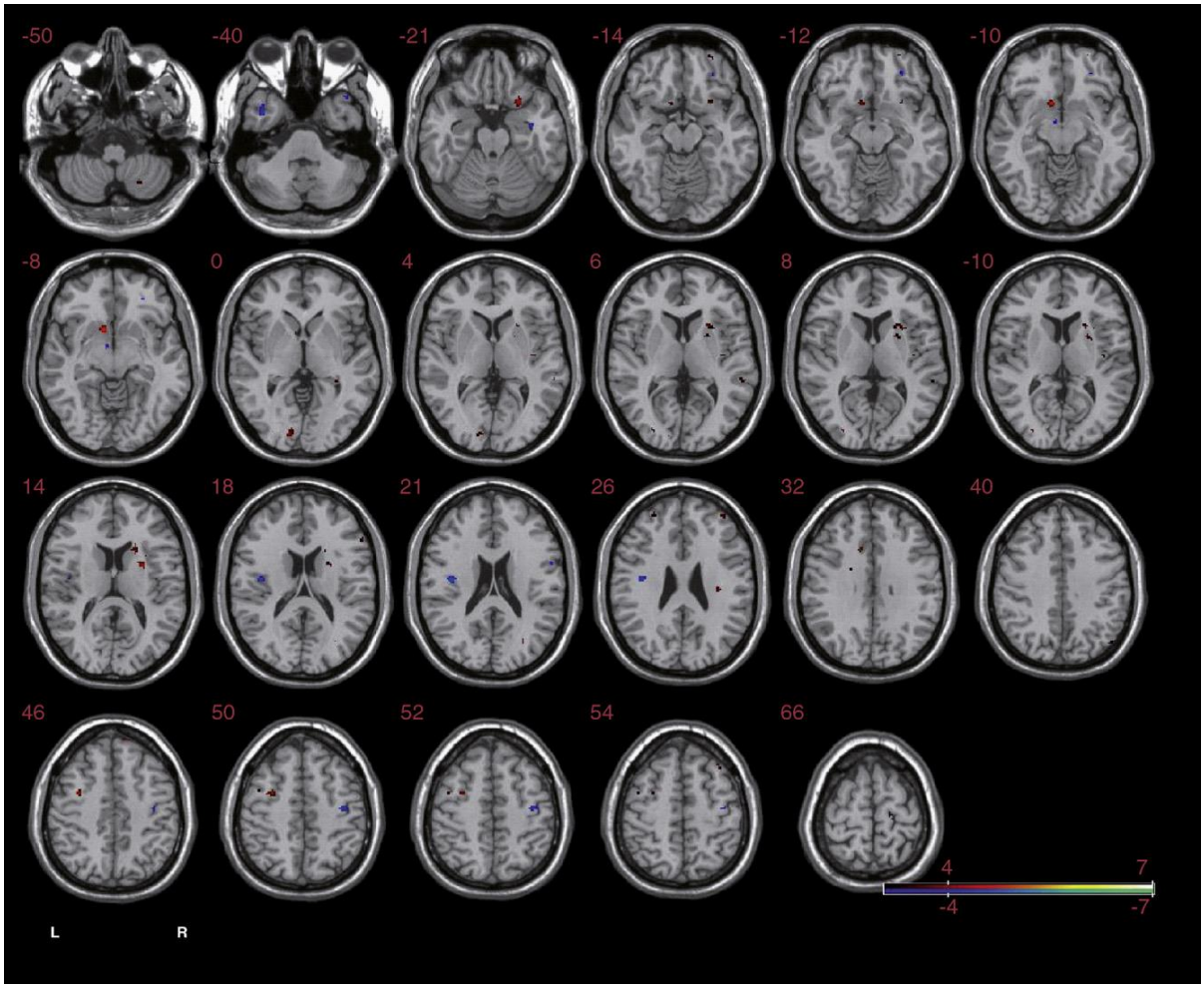

Figure 5 Effect of 2-week pudendal nerve stimulation in the four, clinically improved subjects (Bladder filling prior vs. bladder filling following the 2-week pudendal nerve stimulation treatment). Data are shown at a voxel threshold of p uncorrected≤0.001, cluster extent threshold≥10 voxels, corresponding to T≥3.1. Display conventions are identical to those described under **Figure 4** legend.

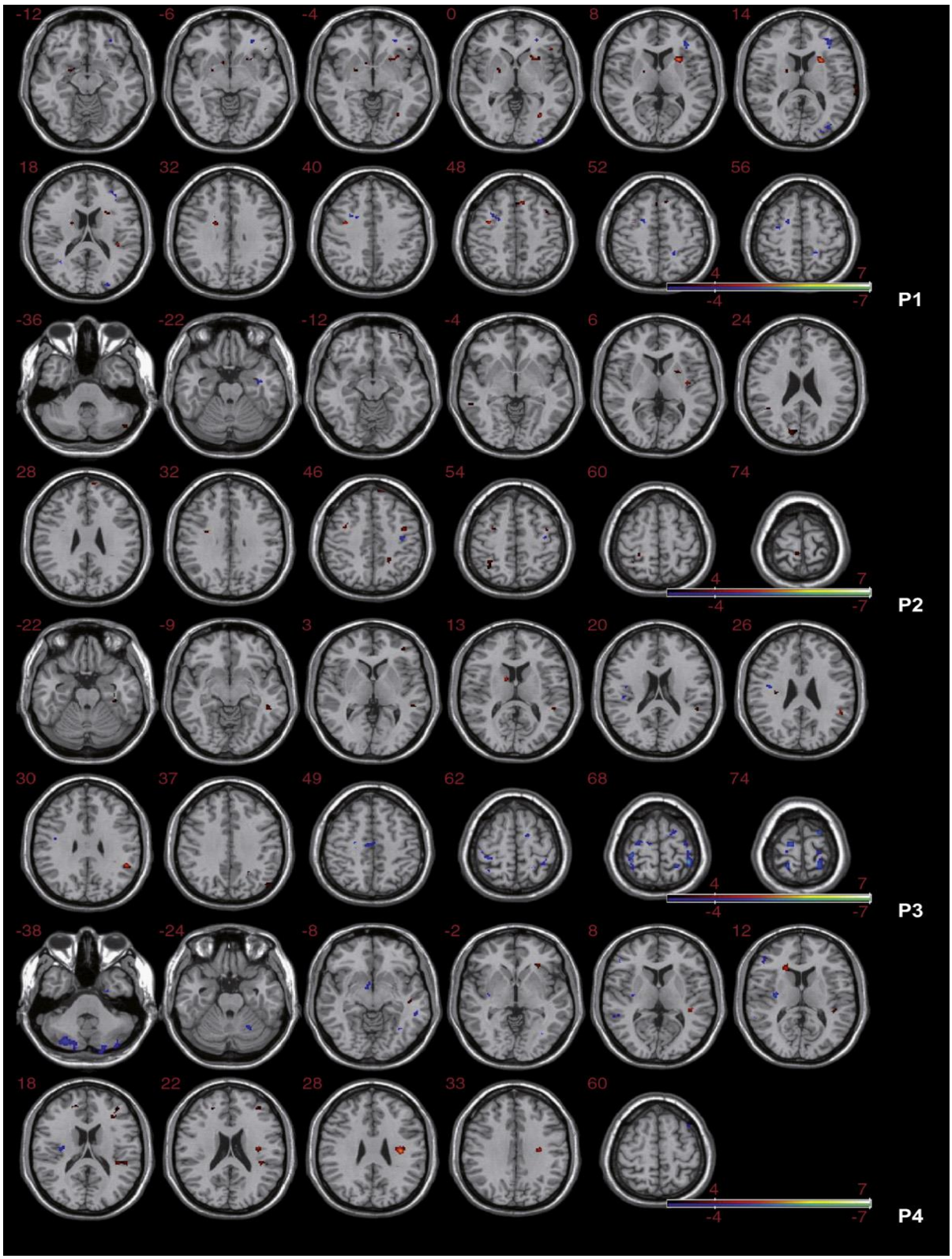

Figure 6 Individual results of the bladder filling prior vs. following 2-week pudendal nerve stimulation treatment in those subjects who showed clinical improvement. Areas are shown in which significant BOLD change is seen in either direction at the voxel threshold of p uncorrected≤0.001 and cluster extent threshold≥20 voxels (corresponding to T≥3.1). Anatomical regions which showed BOLD decrease after the pudendal stimulation treatment are shown in blue shades whereas areas which showed BOLD increase are presented in red shades. MNI normalized, individual T-maps are overlaid on a standard MNI template (MRIcro; Rorden and Brett, 2000); MNI z coordinates of each slice are provided; slices are displayed in neurological convention.

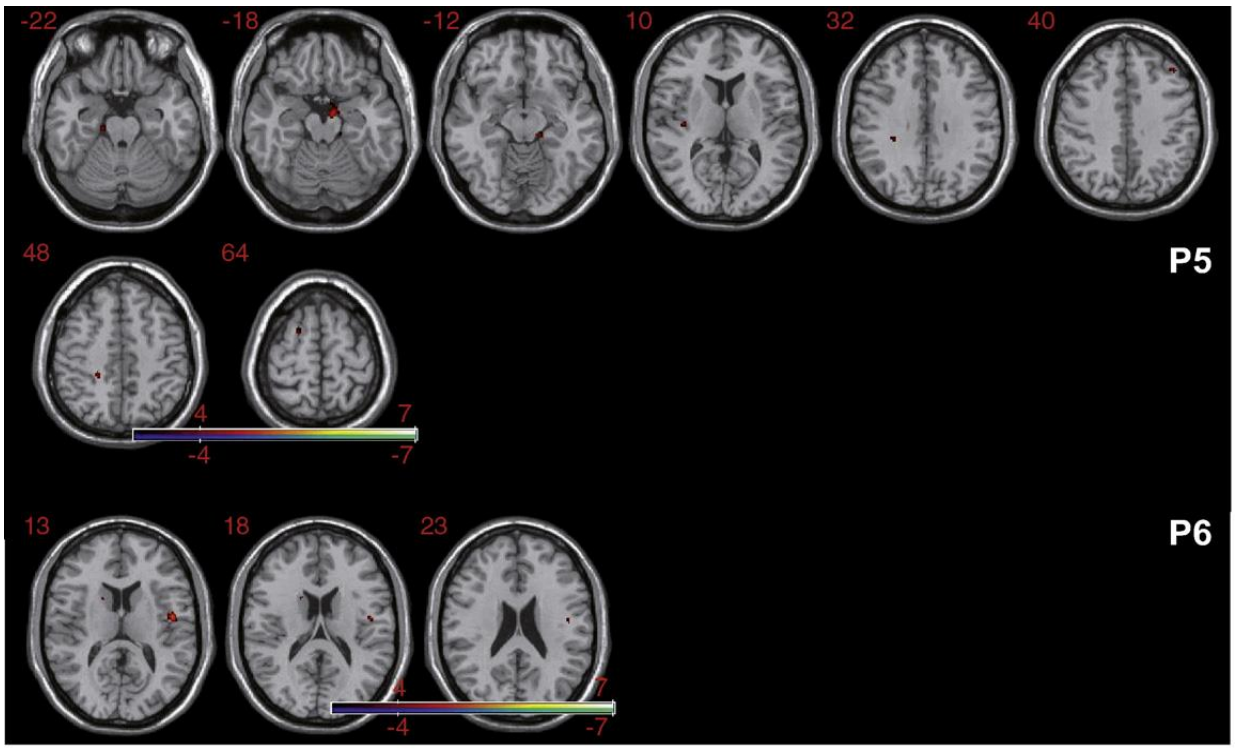

Figure 7 Individual results of the bladder filling prior vs. following 2-week pudendal nerve stimulation treatment in those subjects who did not show clinical change. Areas are shown in which significant BOLD change has been seen in either direction at the voxel threshold of p uncorrected≤0.001 and cluster extent threshold≥20 voxels (corresponding to T≥3.1). Anatomical regions which showed BOLD decrease after the pudendal stimulation treatment are shown in blue shades whereas areas which showed BOLD increase are presented in red shades. MNI normalized, individual T-maps are overlaid on a standard MNI template (MRIcro; Rorden and Brett, 2000); MNI z coordinates of each slice are provided; slices are displayed in neurological convention.

ACUTE NEUROMODULATORY EFFECT OF SIMULTANEOUS PUDENDAL NERVE STIMULATION ON THE NEURAL SUBSTRATE OF BLADDER FILLING SENSATION

The results of the bladder filling combined with pudendal nerve stimulation>rest contrast are shown in **Figure 2** at a voxel threshold of *p* (FDR correction)≤0.01 and kE≥50 voxels (corresponding to T≥3.84 and *p* uncorrected≤0.001); for a detailed list see **Supplementary Table B**.

This contrast revealed activated areas comprising bilaterally the cerebellum (predominantly right) and inferior parietal lobules (BA 40, predominantly right), the left insula and temporo-polar regions (BA 21 and 37) as well as the right temporal pole (BA 38) and inferior frontal gyrus (BA 47).

The results of the direct statistical comparison of the *bladder filling vs. bladder filling with simultaneous pudendal nerve stimulation* conditions in both directions are presented in **Figure 3**; for details see **Supplementary Table C**. This contrast elicited less robust activation than the filling versus rest conditions so the results are presented at a slightly less strict voxel threshold of *p* uncorrected≤0.001, corresponding to T≥3.1 and kE≥10 voxels.

Decreased activation was seen in the right insula (a posterior superior region), precentral gyri bilaterally (predominantly right), left middle frontal gyrus, right temporo-polar and hippocampal regions

and occipital lobes bilaterally. The BOLD signal increased in the right insula (a posterior inferior region), left cerebellum and in the left posterior middle temporal gyrus (BA 21).

SHORT-TERM NEUROMODULATORY EFFECT OF PUDENDAL NERVE STIMULATION (CONTRAST BETWEEN BLADDER FILLING PRIOR VS. FOLLOWING THE 2-WEEK STIMULATION TRAINING)

This contrast has been evaluated in all six participants as well as separately in those four subjects, who showed clinical change following the 2-week stimulation therapy (**Table 1B**). Both group results are presented at a voxel thresholds of *p* uncorrected≤0.001, corresponding to T≥3.1 and kE≥10 voxels.

As can be seen in **Figure 4** (for detailed results see **Supplementary Table D**), most areas showed increased activation following treatment: bilateral caudate, right insula (BA 13), right frontal lobe (orbito-frontal, BA 47; middle and superior frontal gyri, BA 8, 9 and 6), cingulate gyrus (BA 24 and 31), left occipital gyrus (BA 18), left parahippocampal gyrus (BA 19), right middle temporal gyrus, right cerebellum and the right inferior parietal lobule (BA 39 and 40). Activation decrease was seen in only two areas: right precentral gyrus (BA 4) and left temporo-polar region (inferior temporal gyrus; BA 21).

The *clinical and urodynamical data* after the short-term neuromodulatory effect of pudendal nerve stimulation are summarized in **Table 1**. Two of the six trained participants did not notice any change in their bladder sensation or voiding control after treatment (p5 and p6 in **Table 1A**). Four subjects experienced clinical improvement such as improved bladder sensation, increased reflex capacity and improved voluntary voiding, and easier CISC due to decreased sphincter spasticity (**Table 1B**). Based on urodynamic examinations, these subjects showed e.g. enhanced bladder compliance or a desire to void sensation. The group fMRI analysis of the *four clinically improved* patients is shown in **Figure 5** and in **Supplementary Table E**.

In these four subjects, the activation enhancement was more prominent following the treatment; this tendency was seen in the caudate bilaterally, right insula, right inferior (BA 46, 47), middle (BA 10) and superior (BA 8) frontal gyri as well as precentral gyrus (BA 4), anterior cingulate (BA 24, 32), right cerebellum, occipital lobe bilaterally (BA 17 and 18), right inferior parietal lobule (BA 40), right superior temporal gyrus (BA 22) and right hippocampal/parahippocampal region. Note that these areas also showed BOLD increase when analyzed in all six participants. Additionally, enhanced activation was seen in the right putamen and the left precentral (BA 6) and middle frontal (BA 9) gyri. Activation decreased following treatment in the right precentral gyrus (BA 4 and 6) and left temporo-polar region (BA 21 and 38). These areas showed similar tendency in all six participants irrespective of clinical outcome. Additionally, areas showing decreased activation included the left insula (BA 13), right temporo-polar region (BA 20 and 38), right hippocampus, left pallidum and right inferior frontal gyrus (BA 11).

The *single subject results* are summarized in **Figure 6** and **Figure 7** at a threshold of *p* uncorrected≤0.001, corresponding to T≥3.1 and kE≥20 voxels. The individual data have been MNI normalized and overlaid on the standard MNI template. The main finding from the single subject analysis is that the four subjects with clinical improvement showed a similar activation pattern as in the

group analysis though with large variability; the two subjects without clinical improvement on the other hand showed no change to the pretreatment neural pattern.

RESULTS OF THE REGION OF INTERES ANALYSIS

Results of the region of interest analysis are shown in **Figure 8** at a threshold of *p* uncorrected≤0.005, corresponding to T≥2.6 and kE≥3 voxels. The figure shows the brainstem sections that most probably correspond to the anatomical location of the pontine micturition/continence centers and the PAG, at which significant clusters have been found in our analyses. Since **Figure 8** is selfexplanatory in terms of results, the potential relevance of the different regions will be discussed under Discussion.

DISCUSSION

In the present study, we report the central correlates of bladder filling representation in patients with SCI and investigate the potential neuromodulatory effect of simultaneous pudendal nerve stimulation as well as the effects of rehabilitation treatment using electrical pudendal nerve stimulation for 2 weeks. The main findings of our study are summarized as follows. (i) The *central neural substrate of bladder filling sensation* is essentially maintained in incomplete SCI patients at least in the subacute stage following the injury. However, differences are also present as compared to findings reported in healthy subjects [1, 2, 5, 7-13, 35]. Right prefrontal activation is more prominent in the left hemisphere as opposed to the right hemisphere in healthy subjects, which may indicate the loss of inhibitory input normally arriving from the right frontal lobe accompanied by a compensatory, or decompensatory, increased contralateral involvement. Insular and inferior frontal gyrus activations are also slightly left dominant as opposed to the right dominance in healthy subjects. (ii) *Acute pudendal nerve stimulation* had a central neuromodulatory effect, primarily inhibitory. In the right insula, a region implicated in homeostatic interoception in humans, signal increase was seen in an inferior posterior area and signal decrease in a superior posterior region. (iii) *The 2-week pudendal nerve stimulation treatment also induced neural reorganization*, manifesting primarily as enhanced activation in regions implicated in the cortical control of LUT function. Our results also suggest a correlation between clinical improvement of the LUT function and the neuromodulatory effect of the pudendal stimulation treatment. In the following sections, we discuss these findings in more detail.

CENTRAL NEURAL REPRESENTATION OF BLADDER FILLING SENSATION IN INCOMPLETE SCI PATIENTS

Passive filling of the bladder elicited a fairly robust activation in an extensive neural network in the SCI patient group. Most of the activated areas have been implicated in the normal sensation of increasing bladder filling. These included the cerebellum [7, 8, 35], the insula, predominantly on the right, [7-9, 11, 12, 35], the right inferior frontal gyrus [7, 10, 12, 35], the thalamus [8, 9], the anterior cingulate gyrus [7-10, 12, 13, 35], the right middle temporal gyrus [35] and the inferior parietal lobule [35]. The

current patient data are also similar to our previous observations in healthy volunteers using the same stimulation paradigm [35].

The recruitment of a similar network in healthy subjects and SCI patients suggests that the cortical substrate of bladder sensation in SCI patients is maintained, at least in the subacute stage following injury as it was the case in most patients (average time since injury 8.2 months). However, differences in activation patterns between healthy and SCI participants have also been observed that are worth discussing. For example, in SCI patients we see a clear left dominant activation of prefrontal areas, whereas healthy subjects elicited predominantly right prefrontal activations during passive bladder filling paradigms [10, 35]. Moreover, activations in the insula and inferior frontal gyrus were bilateral with left dominance in our patients, whereas more explicit in the right hemisphere in healthy subjects. The right insula is implicated in homeostatic interoception in humans [36], whereas the right inferior frontal gyrus has been implicated in the sensation of bladder fullness [9-13] as well as in deciding when and where micturition is appropriate [1, 5]. In line with this suggestion, Griffiths et al. [8] found that patients with poor bladder control display weak orbitofrontal activation even at larger bladder volumes. Moreover, in a previous fMRI study investigating the areas responsible for continence [14], we demonstrated that voluntary inhibition of micturition activates frontal areas bilaterally with a prominent right dominance. Therefore, the weaker right frontal activation in the SCI patients may reflect decreased sensation of bladder fullness and/or missing inhibitory input normally arriving from the right frontal lobe resulting in detrusor overactivity. In this context, the left dominant prefrontal activation in the SCI patients may be interpreted as compensatory or decompensatory reorganization. It is noteworthy that reversed lateralization of activation was also observed in other brain areas such as the precuneus (right in the patients, left in healthy subjects), the putamen (bilateral in patients, right in healthy subjects) and the cerebellum (right dominant in patients, left dominant in healthy subjects).

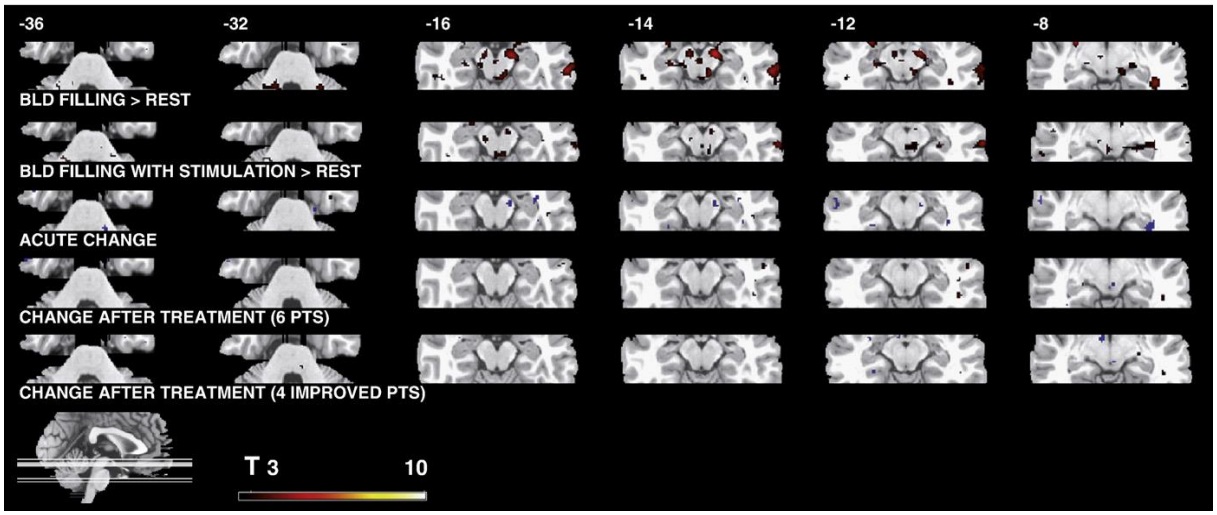

Figure 8 Results of the region of interest analysis in the pons and midbrain of each contrast. Areas are shown at the voxel threshold of p uncorrected≤0.005 and cluster extent threshold≥3 voxels (corresponding to T≥2.6). The contrasts of the changes have been evaluated in both directions. Anatomical regions which showed BOLD decrease are shown in blue shades whereas areas which showed BOLD increase are presented in red shades. MNI normalized, group analysis T-maps are overlaid on a standard MNI template (MRIcro; Rorden and Brett, 2000); MNI z coordinates of each slice are provided; slices are displayed in neurological convention

ACUTE NEUROMODULATORY EFFECT OF PUDENDAL NERVE STIMULATION

Simultaneous pudendal nerve stimulation changed the neural pattern of bladder filling sensation. Fewer brain areas were activated and they showed a weaker BOLD response. This suggests that pudendal nerve stimulation in SCI patients had an acute, central neuromodulatory effect on the neural substrate of bladder filling sensation, which was primarily inhibitory. The signal decreased in the right insula (a posterior superior region), precentral gyri bilaterally (predominantly right), left middle frontal gyrus, right temporo-polar region, right hippocampus and occipital lobe. This inhibitory effect of simultaneous pudendal stimulation during bladder filling is similar to our previous observations in healthy subjects [35], though the areas deactivating are only partially overlapping; i.e. in bilateral frontal and prefrontal regions, right anterior cingulate and right putamen in the healthy study. Activation enhancement was also observed in the right posterior inferior insula, left cerebellum and left middle temporal gyrus in the SCI patients. Similarly, healthy subjects also showed BOLD increase in the postero-ventral right insula during combined stimulation.

As already mentioned above, the right insula is implicated in homeostatic interoception in humans, as evinced by recent neuroimaging findings [36]. The direction of affective information transfer within the insula is postero-anterior: tracts conveying contralateral homeostatic afferent input project to the mid/posterior dorsal insula, which is then re-represented in the anterior insula before reaching the right orbito-frontal region; this information flow is considered to be crucial for the subjective and emotional evaluation of a physical sensation. In this context, it is not surprising that pudendal stimulation, a visceral sensation with a potential affective component, leads to increased BOLD response in the right insula even though the overall stimulation effect has been inhibitory. The simultaneous signal decrease on a more dorsal region of the right posterior insula on the other hand may simply reflect a compensatory vascular response to the increased flow in the ventral insula.

NEUROMODULATORY EFFECT OF 2-WEEK REHABILITATION USING PUDENDAL NERVE STIMULATION

Following the 2-week pudendal stimulation treatment, the six patients showed significant hemodynamic response changes in several brain areas, supporting the hypothesis that peripheral pudendal nerve stimulation does not only act on peripheral nerve collaterals, but also induces changes in the activation pattern of central cortical and subcortical structures.

Similar to the acute effect, the 2-week treatment elicited increases as well as decreases in the BOLD response compared to the activation pattern before treatment. However, the most prominent effect was activation enhancement, observed in the right insula, right frontal lobe (inferior and middle frontal gyri, precentral gyrus and supplementary motor area), anterior cingulate, right cerebellum, right inferior parietal lobule, bilateral caudate nucleus and left occipital lobe. Since most of these areas are implicated in controlling LUT function, this finding suggests that training with pudendal stimulation in the SCI patients increases activation in the normal neural substrate of LUT control (the occipital lobe is not typical for bladder control, but Griffiths et al. [8] report bilateral occipito-parietal regions). We did not find activation of motor (control) regions, most probably because our fMRI task did not include

voiding or pelvic floor muscle contractions/relaxations and was thus a passive rather than an active task. However, the cerebellum which might be involved in subtle motor control and coordination of the LUT [2, 37] showed increased activity after the stimulation training.

All subjects indicated “no” or only faint bladder sensations before pudendal stimulation (**Table 1A**). Group analysis including only the *four subjects who showed clinical improvement* (**Table 1B**) demonstrated that fMRI findings were neither less significant nor less extensive to the results from all six subjects (**Supplementary Table E**). On the contrary, it appeared that excluding the two participants, who did not show clinical change, increased the statistical power probably due to reduced noise of the group data (see also the discussion of the individual results below). BOLD signal increase was again observed in areas implicated in normal LUT control including the right insula, basal ganglia, bilateral prefrontal areas with right dominance, anterior cingulate, right cerebellum, right temporal lobe (similar region to that found by Mehnert et al., [35]), right inferior parietal lobule and occipital lobe bilaterally. Specifically, the two subjects with no clinical improvement did not show BOLD signal increase in frontal areas (data not shown).

BOLD decrease was also seen in several areas in both group analyses (see the Results sections for details); from these areas we would point out the right amygdala, which showed this change only in those four subjects who improved clinically. The right amygdala is implicated in emotional processing, especially when the emotional state is negative. A diminution of the unpleasant visceral sensation arriving from the bladder in the paraplegic patients following the stimulation treatment may explain the decreased activation in this region.

Due to the relatively low number of the participants, we performed fixed-effects group analysis, which does not account for individual variance. Therefore, we also carried out individual analyses that revealed fairly large variance between the different subjects, which is not unusual in patient fMRI studies. Although we do not have the possibility to statistically support our claim, the qualitative evaluation of the individual data sets suggests that the BOLD signal changes were more prominent in those four subjects who showed clinical improvement and less prominent in the two subjects without clinical improvement (**Table 1B**). This claim is in fact supported by the group statistics as well, since adding the latter two data sets to the analysis has reduced the statistical power instead of increasing it (see above). It is also noteworthy that the two subjects without clinical improvement included a patient in chronic SCI stage and a patient classified as ASIA complete but with preserved bladder sensation. Therefore, we argue that the results of our pilot experiment in terms of the 2-week pudendal stimulation might indicate a potential correlation between clinical improvement and central neural pattern change in four incomplete, subacute SCI patients, especially because these enhanced activations occurred in brain areas implicated in the normal LUT control. However, due to the subacute stage of the four clinically improved patients (mean duration since injury 1.73 months, median 1.65 months, range 0.8–2.8 months) we cannot exclude that some degree of spontaneous clinical improvement would also play a role.

Future studies with a larger patient population should be performed to investigate more in detail the effects of pudendal nerve stimulation in patients with similar neurological deficits. For example, this treatment could be applied in MS patients that have bladder dysfunctions and represent a suitable

population in fMRI studies. This investigation will then allow more specifically drawing conclusions concerning the specific role of brain regions involved in the desire to void sensation or other, bladder-related sensations.

REGION OF INTEREST ANALYSIS OF THE PONS AND MIDBRAIN IN THE DIFFERENT CONTRASTS

The bladder filling>rest contrast in the eight SCI participants elicited clear activation in the PAG, and in a continuous area in the right midbrain. The involvement of the PAG is expected in this type of paradigm since the region is responsible for transferring sensory information from the bladder towards higher cortical centers. Activations in the pons were less clear. In the left pons, an area is activated, which would logically correspond to the M region or pontine micturition center since normally the M-region responds to increasing levels of bladder filling. However, anatomically this region corresponds better to the L region as it is fairly laterally localized. If this is the case, a very tentative explanation could be that in SCI patients increasing levels of bladder filling may lead to increased response in the pontine continence center thus, potentially contributing to the occurrence of negative detrusor-sphincter synergy.

During bladder filling and simultaneous pudendal nerve stimulation (acute neuromodulatory effect) the PAG activation was prominent and fairly symmetrically localized around the aqueduct. It is noteworthy that other regions of the midbrain, i.e. substantia nigra, which are normally not implicated in the neural substrate of bladder sensation but showed activation in the bladder filling experiment, became less activated during the combined stimulation, i.e. the significantly deactivated region in the right cerebral peduncule. This may tentatively be interpreted as a neural pattern normalization effect of the stimulation as the activations became more focused in the normally implicated region. In this contrast, the significant voxels in the pons are too laterally localized to be interpreted as the pontine M or L regions and most likely represent artifacts.

Following 2 weeks of pudendal stimulation treatment, PAG activation has decreased in all six participants as well as in the four clinically improved subjects. We propose that the PAG may be overactive in the SCI group, potentially due to a decompensatory mechanism following the sudden loss of the spinal afferent input, and that the pudendal stimulation treatment, i.e. increased afferent input, may normalize the PAG activation. The four clinically improved patients additionally showed increased activity in a right pontine region corresponding to the pontine M region. This may again suggest neural pattern normalization since increased levels of bladder tension are expected to elicit M-region activity.

CONCLUSION

Our results suggest that the central neural substrate of bladder filling sensation in incomplete SCI patients is essentially maintained in the subacute stage following the injury. The main difference, as

compared to healthy volunteers, is a decreased right prefrontal activation with concomitant enhanced left prefrontal activation, which suggests that the detrusor overactivity may potentially be the result of the missing inhibitory input normally arriving from the right frontal lobe. The left dominant prefrontal activation in the SCI patients may therefore be interpreted as compensatory or decompensatory reorganization. Reversed lateralization of activation as compared to the normal pattern was also present in other brain areas of the SCI patients.

Acute pudendal nerve stimulation had a central neuromodulatory effect on the neural substrate of bladder filling sensation in SCI patients, which was primarily inhibitory, similar to the effect of this stimulation in healthy subjects. This effect may contribute to the clinically observed efficacy of pudendal stimulation in conditions such as detrusor overactivity. Similar to the healthy results, however, stimulation elicited BOLD signal increase in the right posterior ventral insula, which is a central region for the affective evaluation of visceral interoception in humans. The 2-week pudendal nerve stimulation also induced neural reorganization, supporting the hypothesis that treatment with peripheral pudendal nerve stimulation does have a central effect as well. As opposed to the acute stimulation, the most prominent effect was an enhanced activation. The group analysis from six participants, including four showing clinical improvement in the LUT status, as well as the individual data analyses indicates that the vast majority of the positive BOLD changes are seen in regions implicated in normal LUT control suggesting correlation between the neuromodulatory effect of the pudendal stimulation treatment and the clinical improvement.

FUNDING

This work was supported by the International Institute for Research in Paraplegia (IRP-CH-020/06 ‘Reorganization of cortical representation in somatosensory and autonomic brain areas related to bladder control in spinal cord injured patients’); National Center of Competence in Research (P7 ‘Spinal cord repair’); and Swiss National Science Foundation (320000-113644 ‘Urodynamical and electrophysiological assessment of normal and impaired human bladder function’).

ACKNOWLEDGEMENTS

We would like to thank Jonas Svensson for his involvement in creating the experimental paradigm, Roger Luechinger for helping us at scanning, Remco Renken for the batch scripts used for automating many aspects of the data analysis and Armin Blickenstorfer for IT support.

REFERENCES

1. Griffiths DJ. Cerebral control of bladder function. Curr Urol Rep. 2004;5(5):348-52.

2. Blok BF. Central pathways controlling micturition and urinary continence. Urology. 2002;59(5 Suppl 1):13-7.

3. Blok BF, Holstege G. The central nervous system control of micturition in cats and humans. Behav Brain Res. 1998;92(2):119-25.

4. Holstege G, Griffiths D, de Wall H, Dalm E. Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in the cat. J Comp Neurol. 1986;250(4):449-61.

5. Kavia RB, Dasgupta R, Fowler CJ. Functional imaging and the central control of the bladder. J Comp Neurol. 2005;493(1):27-32.

6. Holstege G. Micturition and the soul. J Comp Neurol. 2005;493(1):15-20.

7. Griffiths D, Tadic SD, Schaefer W, Resnick NM. Cerebral control of the bladder in normal and urge-incontinent women. Neuroimage. 2007;37(1):1-7.

8. Griffiths D, Derbyshire S, Stenger A, Resnick N. Brain control of normal and overactive bladder. J Urol. 2005;174(5):1862-7.

9. Matsuura S, Kakizaki H, Mitsui T, Shiga T, Tamaki N, Koyanagi T. Human brain region response to distention or cold stimulation of the bladder: a positron emission tomography study. J Urol. 2002;168(5):2035-9.

10. Athwal BS, Berkley KJ, Hussain I, Brennan A, Craggs M, Sakakibara R, et al. Brain responses to changes in bladder volume and urge to void in healthy men. Brain. 2001;124(Pt 2):369-77.

11. Nour S, Svarer C, Kristensen JK, Paulson OB, Law I. Cerebral activation during micturition in normal men. Brain. 2000;123 (Pt 4):781-9.

12. Blok BF, Sturms LM, Holstege G. Brain activation during micturition in women. Brain. 1998;121 (Pt 11):2033-42.

13. Blok BF, Willemsen AT, Holstege G. A PET study on brain control of micturition in humans. Brain. 1997;120 (Pt 1):111-21.

14. Zhang H, Reitz A, Kollias S, Summers P, Curt A, Schurch B. An fMRI study of the role of suprapontine brain structures in the voluntary voiding control induced by pelvic floor contraction. Neuroimage. 2005;24(1):174-80.

15. de Groat WC. A neurologic basis for the overactive bladder. Urology. 1997;50(6A Suppl):36-52; discussion 3-6.

16. Tai C, Roppolo JR, de Groat WC. Spinal reflex control of micturition after spinal cord injury. Restor Neurol Neurosci. 2006;24(2):69-78.

17. Schurch B, Schmid DM, Karsenty G, Reitz A. Can neurologic examination predict type of detrusor sphincter-dyssynergia in patients with spinal cord injury? Urology. 2005;65(2):243-6.

18. Burns AS, Rivas DA, Ditunno JF. The management of neurogenic bladder and sexual dysfunction after spinal cord injury. Spine (Phila Pa 1976). 2001;26(24 Suppl):S129-36.

19. Jamil F. Towards a catheter free status in neurogenic bladder dysfunction: a review of bladder management options in spinal cord injury (SCI). Spinal Cord. 2001;39(7):355-61.

20. Reitz A, Schmid DM, Curt A, Knapp PA, Schurch B. Afferent fibers of the pudendal nerve modulate sympathetic neurons controlling the bladder neck. Neurourol Urodyn. 2003;22(6):597-601.

21. Brubaker L. Electrical stimulation in overactive bladder. Urology. 2000;55(5A Suppl):17-23; discussion 31-2.

22. Craggs M, McFarlane J. Neuromodulation of the lower urinary tract. Exp Physiol. 1999;84(1):149-60.

23. Fall M, Lindstrom S. Electrical stimulation. A physiologic approach to the treatment of urinary incontinence. Urol Clin North Am. 1991;18(2):393-407.

24. Tai C, Wang J, Wang X, de Groat WC, Roppolo JR. Bladder inhibition or voiding induced by pudendal nerve stimulation in chronic spinal cord injured cats. Neurourol Urodyn. 2007;26(4):570-7.

25. Hayes KC, Bassett-Spiers K, Das R, Ethans KD, Kagan C, Kramer JL, et al. Research priorities for urological care following spinal cord injury: recommendations of an expert panel. Can J Urol. 2007;14(1):3416-23.

26. Yoo PB, Klein SM, Grafstein NH, Horvath EE, Amundsen CL, Webster GD, et al. Pudendal nerve stimulation evokes reflex bladder contractions in persons with chronic spinal cord injury. Neurourol Urodyn. 2007;26(7):1020-3.

27. Lee YH, Creasey GH, Lim H, Song J, Song K, Kim J. Detrusor and blood pressure responses to dorsal penile nerve stimulation during hyperreflexic contraction of the bladder in patients with cervical cord injury. Arch Phys Med Rehabil. 2003;84(1):136-40.

28. Reitz A, Schmid DM, Curt A, Knapp PA, Schurch B. Autonomic dysreflexia in response to pudendal nerve stimulation. Spinal Cord. 2003;41(10):539-42.

29. Previnaire JG, Soler JM, Perrigot M. Is there a place for pudendal nerve maximal electrical stimulation for the treatment of detrusor hyperreflexia in spinal cord injury patients? Spinal Cord. 1998;36(2):100-3.

30. Wienbruch C, Candia V, Svensson J, Kleiser R, Kollias SS. A portable and low-cost fMRI compatible pneumatic system for the investigation of the somatosensensory system in clinical and research environments. Neurosci Lett. 2006;398(3):183-8.

31. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage. 2002;15(1):273-89.

32. Brett M, Leff AP, Rorden C, Ashburner J. Spatial normalization of brain images with focal lesions using cost function masking. Neuroimage. 2001;14(2):486-500.

33. Lancaster JL, Tordesillas-Gutierrez D, Martinez M, Salinas F, Evans A, Zilles K, et al. Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. Hum Brain Mapp. 2007;28(11):1194-205.

34. Rorden C, Brett M. Stereotaxic display of brain lesions. Behav Neurol. 2000;12(4):191-200.

35. Mehnert U, Boy S, Svensson J, Michels L, Reitz A, Candia V, et al. Brain activation in response to bladder filling and simultaneous stimulation of the dorsal clitoral nerve--an fMRI study in healthy women. Neuroimage. 2008;41(3):682-9.

36. Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci. 2002;3(8):655-66.

37. Griffiths D, Tadic SD. Bladder control, urgency, and urge incontinence: evidence from functional brain imaging. Neurourol Urodyn. 2008;27(6):466-74.

38. Talairach J, Tournoux P. Co-planar Atlas of the Human Brain. Thieme Medical Publishers, New York. 1988.

SUPPLEMENTARY DATA

Supplementary Table A: The neural substrate of bladder filling sensation. Bladder filling > rest contrast, 1st fMRI session, before treatment, eight subjects. Areas were significant at a threshold of p (FDR correction) ≤ 0.01 and extent threshold ≥ 50 voxels (corresponding to T ≥ 3.43 and p voxel level uncorrected ≤ 0.000). Areas are grouped according to anatomical regions. Extent of activation (kE), level of significance (T), MNI and Talairach & Tournoux coordinates of the activation maximum as well as the Brodmann area (BA) are indicated for each cluster

kE	T	*MNI*			*TAL (icbm)*			*TAL (mni2tal)*			Brain area	BA
		x	*y*	*z*	*x*	*y*	*z*	*x*	y	*z*		
2268	6.95	*48*	*−54*	*−42*	*44*	*-49*	*-37*	*48*	*-54*	*-33*	Cerebellum	
	6.29	*38*	*−66*	*−28*	*34*	*-61*	*-26*	*38*	*-65*	*-20*	Cerebellum	
	6.17	*−6*	*−86*	*−18*	*-7*	*-80*	*-20*	*-6*	*-84*	*-11*	Cerebellum	
66	4.62	*0*	*−62*	*−10*	*-1*	*-59*	*-10*	*0*	*-60*	*-5*	Cerebellum (Vermis)	
	3.88	*8*	*−68*	*−16*	*6*	*-64*	*-16*	*8*	*-67*	*-10*	Cerebellum (Vermis)	
	3.76	*2*	*−80*	*−10*	*1*	*-75*	*-12*	*2*	*-78*	*-5*	Cerebellum (Vermis)	
765	6.34	*0*	*−44*	*4*	*-1*	*-43*	*4*	*0*	*-42*	*6*	Cerebellum (Vermis)	
	5.95	*2*	*−10*	*10*	*1*	*-12*	*12*	*2*	*-9*	*10*	Thalamus R	
	5.78	*−2*	*−28*	*10*	*-3*	*-29*	*11*	*-2*	*-27*	*11*	Thalamus L	
160	5.9	*34*	*16*	*−8*	*31*	*14*	*-1*	*34*	*15*	*-7*	Insula R	13
	5.02	*36*	*18*	*−16*	*33*	*16*	*-8*	*36*	*17*	*-14*	Frontal Inf. R	47
	4.2	*26*	*18*	*2*	*23*	*15*	*8*	*26*	*18*	*1*	Putamen R	
176	5.63	*−42*	*24*	*−8*	*-40*	*21*	*-2*	*-42*	*23*	*-8*	Insula L/IFG orb	13 \ 47
	5.32	*−32*	*26*	*−10*	*-30*	*23*	*-3*	*-32*	*25*	*-10*	Insula L/IFG orb	13 \ 47
253	5.43	*−30*	*−2*	*−10*	*-29*	*-3*	*-5*	*-30*	*-2*	*-8*	Putamen L	
	5.1	*−20*	*−18*	*22*	*-20*	*-20*	*22*	*-20*	*-16*	*21*	Caudate L	
	4.66	*−26*	*−22*	*14*	*-25*	*-23*	*14*	*-26*	*-21*	*14*	Thalamus L	
392	5.79	*−36*	*52*	*18*	*-34*	*45*	*24*	*-36*	*51*	*14*	Frontal Mid L	10
	5.69	*−30*	*36*	*14*	*-29*	*31*	*19*	*-30*	*36*	*11*	Frontal Mid L	10
	4.76	*−26*	*56*	*22*	*-25*	*49*	*28*	*-26*	*55*	*17*	Frontal Sup L	9

208	4.74	−48	20	42	-46	13	43	-48	21	38	Frontal Mid L	8
	4.52	−42	6	50	-40	0	49	-42	8	46	Frontal Mid L	6
	4.51	−40	6	40	-38	1	40	-40	8	36	Precentral L	6
213	5.88	28	16	22	25	11	26	28	17	19	Anterior Cingulate	24
	5.12	30	16	30	26	10	33	30	17	27	Frontal Med /AC R	32
	4.98	38	14	20	34	9	24	38	14	18	Frontal Inf /AC R	24
138	5.05	0	34	46	-1	26	48	0	35	41	Fro. Sup Med. SMA	8
	4.51	−2	32	36	-3	25	39	-2	33	32	Anterior Cingulate	32
60	5.1	26	40	4	23	35	12	26	39	2	Ant. Cingulate	32
191	5.72	22	−50	36	19	-52	32	22	-47	36	Precuneus R	7
	4.72	30	−48	22	26	-49	20	30	-45	23	Post. Cingulate	31
	4.62	34	−56	16	30	-55	14	34	-54	17	Temporal Mid R	39
129	5.96	−50	−48	32	-48	-49	28	-50	-45	32	Supramarginal L	40
50	5.61	12	−12	−14	10	-12	-9	12	-12	-11	Midbrain (Subst.Nig.)	
63	5.37	32	6	−36	29	7	-27	32	4	-30	Temporal Pole Sup R	38
	4.59	38	4	−30	35	4	-22	38	3	-25	Temporal Pole Sup R	38
61	4.84	48	18	−20	44	17	-11	48	17	-18	Temporal Pole Sup R	38
	3.84	46	24	−12	42	21	-4	46	23	-11	Frontal Inf Orb R	47
77	4.86	62	−26	−16	56	-25	-12	61	-26	-12	Temporal Mid R	20
	3.75	68	−32	−12	62	-31	-8	67	-32	-9	Temporal Mid R	21

Supplementary Table B: Acute effect of simultaneous pudendal nerve stimulation on the neural substrate of bladder filling sensation. Bladder filling with simultaneous pudendal nerve stimulation > rest contrast, 1st fMRI session, eight subjects. Areas were significant at a threshold of p (FDR correction) ≤ 0.01 and extent threshold ≥ 50 voxels (corresponding to T ≥ 3.84 and p voxel level uncorrected ≤ 0.000). Areas are grouped according to anatomical regions. Extent of activation (kE), level of significance (T), MNI and Talairach & Tournoux coordinates of the activation maximum as well as the Brodmann area (BA) are indicated for each cluster.

kE	T	*MNI*			*TAL (icbm)*			*TAL (mni2tal)*			Brain area	BA
		x	*y*	*z*	*x*	*y*	*z*	*x*	y	*z*		
105	5.86	*34*	*−60*	*−28*	*31*	*-55*	*-26*	*34*	*-59*	*-21*	Cerebellum	
	5.64	*28*	*−64*	*−24*	*25*	*-59*	*-23*	*28*	*-63*	*-17*	Cerebellum	
185	5.77	*14*	*−82*	*−34*	*12*	*-75*	*-33*	*14*	*-81*	*-25*	Cerebellum	
	5.05	*16*	*−84*	*−46*	*14*	*-76*	*-44*	*16*	*-83*	*-35*	Cerebellum	
	4.04	*12*	*−80*	*−24*	*10*	*-74*	*-24*	*12*	*-79*	*-16*	Cerebellum	
54	5.66	*0*	*−42*	*2*	*-1*	*-41*	*2*	*0*	*-41*	*4*	Cerebellum (Vermis)	
70	4.86	*−14*	*−80*	*−26*	*-14*	*-74*	*-26*	*-14*	*-79*	*-18*	Cerebellum	
114	5.91	*−58*	*−44*	*28*	*-55*	*-45*	*24*	*-57*	*-41*	*28*	Lobulus Parietalis Inf. L	40
	4.85	*−60*	*−40*	*20*	*-57*	*-40*	*17*	*-59*	*-38*	*20*	Temporal_Sup G. L	22
	4.25	*−52*	*−36*	*26*	*-50*	*-37*	*23*	*-51*	*-34*	*26*	Lobulus Parietalis Inf. L	40
299	5.87	*60*	*−34*	*22*	*54*	*-36*	*22*	*59*	*-32*	*22*	Lobulus Parietalis Inf. R	40
	5.56	*50*	*−36*	*22*	*45*	*-37*	*21*	*50*	*-34*	*22*	Lobulus Parietalis Inf. R	40
	5.14	*54*	*−28*	*26*	*49*	*-30*	*26*	*53*	*-26*	*25*	Lobulus Parietalis Inf. R	40
98	5.67	*−62*	*−48*	*2*	*-59*	*-46*	*1*	*-61*	*-46*	*4*	Insula / Temporo-polar L	21
	4.49	*−60*	*−58*	*−12*	*-57*	*-54*	*-13*	*-59*	*-57*	*-7*	Insula / Temporo-polar L	37
	4.21	*−60*	*−60*	*−4*	*-57*	*-57*	*-6*	*-59*	*-58*	*0*	Insula / Temporo-polar L	37
56	5.28	*38*	*24*	*−14*	*34*	*22*	*-6*	*38*	*23*	*-13*	Frontal Inf. Orb. R	47
50	5.14	*50*	*14*	*−14*	*45*	*12*	*-6*	*50*	*13*	*-12*	Temporal Pole Sup G. R	38

Supplementary Table C shows the results of the bladder filling vs. bladder filling with simultaneous pudendal nerve stimulation contrast. Acute pudendal stimulation effect experiment, 1st fMRI session, eight subjects; i.e. Table C is the statistical comparison of the activation patterns shown in Table A and B.Areas are shown in which the simultaneous pudendal nerve stimulation elicited significant change in either direction at the threshold of p uncorrected ≤ 0.001 and extent threshold ≥ 10 voxels (corresponding to T ≥ 3.1). Anatomical regions which showed BOLD-decrease due to the simultaneous pudendal stimulation are listed in the upper part of the table in blue shades whereas areas, which showed BOLD-increase are listed in the lower part of the table in red shades. In the corresponding Figure C, the same 'color code' has been applied. Areas are grouped according to anatomical regions. Extent of activation (kE), level of significance (T), MNI and Talairach & Tournoux coordinates of the activation maximum as well as the Brodmann area (BA) are indicated for each cluster

kE	T	*MNI*			*TAL (icbm)*			*TAL (mni2tal)*			Brain area	BA
		x	*y*	*z*	*x*	*y*	*z*	*x*	y	*z*		
47	5.36	*34*	*−20*	*18*	*30*	*-22*	*19*	*34*	*-19*	*18*	Insula R	13
13	3.46	*−58*	*2*	*24*	*-55*	*-2*	*25*	*-57*	*3*	*22*	Precentral L	6
78	5.27	*36*	*−14*	*52*	*32*	*-20*	*50*	*36*	*-11*	*48*	Precentral R	4
11	3.67	*54*	*2*	*28*	*49*	*-3*	*30*	*53*	*3*	*26*	Precentral R	4
13	3.6	*−32*	*14*	*24*	*-31*	*9*	*26*	*-32*	*15*	*21*	Frontal Mid L	9
	3.47	*−30*	*14*	*32*	*-29*	*9*	*33*	*-30*	*15*	*29*	Frontal Mid L	9
13	3.66	*46*	*10*	*−32*	*42*	*10*	*-23*	*46*	*8*	*-27*	Temporo- polar R	21
31	4.85	*34*	*−10*	*−20*	*31*	*-10*	*-14*	*34*	*-11*	*-16*	Hippocampus R	
16	3.89	*36*	*−32*	*−8*	*32*	*-31*	*-5*	*36*	*-31*	*-5*	Hippocampus R	
11	3.48	*−38*	*−82*	*−6*	*-36*	*-77*	*-9*	*-38*	*-80*	*-1*	Occipital Inf. L	18
20	3.75	*24*	*−84*	*44*	*20*	*-84*	*36*	*24*	*-79*	*45*	Occipital Sup. R	19
100	4.63	*36*	*−12*	*6*	*32*	*-14*	*9*	*36*	*-11*	*6*	Insula R	13
	4.44	*34*	*−10*	*26*	*30*	*-14*	*27*	*34*	*-8*	*24*	Insula R	13
10	4.02	*−38*	*−78*	*−48*	*-36*	*-70*	*-46*	*-38*	*-78*	*-36*	Cerebellum L	
15	3.41	*−48*	*−46*	*0*	*-46*	*-44*	*-1*	*-48*	*-45*	*2*	Temporal Mid. G. L	21

Supplementary Table D: Results of the bladder filling prior vs. bladder filling following two-week pudendal nerve stimulation treatment. Data from the 1st vs. 2nd fMRI session, from all six participants irrespective of their clinical change. Areas are shown in which the two-week pudendal nerve stimulation elicited significant change in either direction at the threshold of p uncorrected ≤ 0.001 and extent threshold ≥ 10 voxels (corresponding to T ≥ 3.1). Anatomical regions which showed BOLD-decrease after the pudendal stimulation treatment are listed in the upper part of the table in blue shades whereas areas, which showed BOLD increase are listed in the lower part of the table in red shades. (In the corresponding Figure D, the same 'color code' has been applied.) Areas are grouped according to anatomical regions. Extent of activation (kE), level of significance (T), MNI and Talairach & Tournoux coordinates of the activation maximum as well as the Brodmann area (BA) are indicated for each cluster.

kE	T	*MNI*			*TAL (icbm)*			*TAL (mni2tal)*			Brain area	BA
		x	*y*	*z*	*x*	*y*	*z*	*x*	y	*z*		
23	4.56	36	-14	50	32	-19	48	36	-11	46	Precentral G R	4-6
28	4.46	*-42*	*4*	*-40*	*-40*	*6*	*-32*	*-42*	*2*	*-34*	Temporal Inf. L	21
50	5.13	*−12*	*14*	*−8*	*-12*	*12*	*-2*	*-12*	*13*	*7*	Caudate L	
94	4.3	*18*	*16*	*20*	*15*	*11*	*24*	*18*	*16*	*18*	Caudate R	
12	4.08	*38*	*−10*	*26*	*34*	*-14*	*27*	*38*	*-8*	*24*	Insula R	13
34	4.03	*38*	*−12*	*10*	*34*	*-14*	*13*	*38*	*-11*	*10*	Insula R	13
40	4.28	*30*	*−22*	*28*	*26*	*-25*	*28*	*30*	*-20*	*27*	Insula R (extens)	
34	4.61	*20*	*16*	*−20*	*18*	*15*	*-12*	*20*	*15*	*-18*	Frontal Inf Orb R	47
24	4.33	*32*	*28*	*54*	*28*	*19*	*55*	*32*	*30*	*48*	Frontal Med R	6
12	3.86	*48*	*28*	*40*	*43*	*21*	*43*	*48*	*29*	*35*	Frontal Mid R	9
10	3.55	*40*	*16*	*54*	*35*	*8*	*54*	*40*	*18*	*49*	Frontal Mid R	8
12	3.48	*16*	*−20*	*64*	*13*	*-26*	*60*	*16*	*-16*	*60*	Precentral R	6
12	3.43	*20*	*12*	*52*	*17*	*5*	*52*	*20*	*14*	*47*	Frontal Sup/Med R	6
13	4.33	*−22*	*0*	*32*	*-22*	*-4*	*32*	*-22*	*1*	*29*	Frontal sup L AC L	
10	3.58	*−34*	*54*	*24*	*-33*	*47*	*30*	*-34*	*53*	*19*	Frontal Mid L/AC	9
29	3.78	*8*	*−48*	*40*	*6*	*-50*	*36*	*8*	*-45*	*39*	Precuneus R/Cingulate	31
11	3.49	*18*	*−10*	*40*	*15*	*-15*	*39*	*18*	*-8*	*37*	Cingulum Mid R	24
10	3.48	*−18*	*−92*	*−2*	*-18*	*-87*	*-6*	*-18*	*-89*	*3*	Occipital Mid L	18
48	4.22	*−42*	*−48*	*−2*	*-40*	*-46*	*-3*	*-42*	*-47*	*1*	Temporal Mid L/Parahipp 19	
13	3.7	*42*	*−54*	*4*	*38*	*-53*	*4*	*42*	*-52*	*6*	Temporal Mid R	

18	3.68	*46*	*−34*	*−12*	*42*	*-33*	*-9*	*46*	*-33*	*-8*	Temporal Mid R/Parahipp	36
	3.41	*46*	*−36*	*−4*	*42*	*-35*	*-2*	*46*	*-35*	*-2*	Temporal Mid R	
26	4.23	*22*	*−62*	*−48*	*20*	*-55*	*-44*	*22*	*-62*	*-37*	Cerebelum R	
13	3.57	*66*	*−32*	*40*	*60*	*-35*	*38*	*65*	*-29*	*38*	Inf Par. Lobule R	40
11	3.45	*44*	*−72*	*40*	*39*	*-73*	*34*	*44*	*-68*	*40*	Inf. Par. Lobule R	39

Supplementary Table E: Effect of two-week pudendal nerve stimulation in the four, clinically improved subjects. Data are shown at a threshold of p uncorrected ≤ 0.001 and extent threshold ≥ 10 voxels corresponding to T ≥ 3.1. Table conventions are the same as described under Table D.

kE	T	*MNI*			*TAL (icbm)*			*TAL (mni2tal)*			Brain area	BA
		x	*y*	*z*	*x*	*y*	*z*	*x*	y	*z*		
76	4.98	−42	−12	24	*-40*	*-15*	*24*	*-42*	*-11*	*23*	Insula L	13
40	4.46	36	−14	50	*32*	*-19*	*48*	*36*	*-11*	*47*	Precentral R	4
13	4.03	54	4	22	*49*	*0*	*25*	*53*	*5*	*20*	Precentral R	6
79	4.87	−38	10	−44	*-36*	*12*	*-35*	*-38*	*8*	*-37*	Temporal Pole Mid L	38
	4.52	−42	4	−40	*-39*	*6*	*-32*	*-42*	*2*	*-34*	Temporal Pole Inf L	21
14	3.84	40	20	−38	*36*	*20*	*-27*	*40*	*18*	*-33*	Temporal Pole Mid R	38
15	4.18	30	−4	−50	*27*	*-1*	*-40*	*30*	*-6*	*-42*	Temporal Pole Fusif R	20
27	4.36	34	−8	−22	*31*	*-7*	*-16*	*34*	*-9*	*-18*	Amygdala R	
10	3.68	−10	−4	−8	*-10*	*-5*	*-4*	*-10*	*-4*	*-7*	Pallidum L	
12	3.53	26	42	−12	*23*	*38*	*-3*	*26*	*40*	*-12*	Frontal Inf Orb R	11
65	4.8	*−12*	*14*	*−10*	*-12*	*12*	*-4*	*-12*	*13*	*-9*	Caudate L	
67	3.95	*22*	*16*	*6*	*19*	*13*	*11*	*22*	*16*	*5*	Caudate R	
	3.87	*18*	*18*	*14*	*16*	*14*	*18*	*18*	*18*	*12*	Caudate R	
	3.11	*28*	*12*	*14*	*25*	*8*	*18*	*28*	*12*	*12*	Insula R	
62	4.39	*26*	*2*	*14*	*23*	*-1*	*17*	*26*	*3*	*13*	Putamen R	

	3.68	20	6	10	17	3	14	20	6	9	Putamen R	
16	3.6	36	−12	4	32	-13	7	36	-11	4	Putamen R	
22	4.44	32	−20	28	28	-23	28	32	-18	27	Insula R	
83	4.52	22	16	−20	20	15	-12	22	15	-18	Frontal Inf Orb R	47
10	3.55	24	60	−14	21	55	-3	24	58	-15	Frontal Inf Orb R	10
15	3.51	54	28	18	49	22	23	53	28	15	Frontal Inf Tri R	46
13	3.99	36	50	26	32	42	32	36	50	21	Frontal Mid R	10
10	3.86	10	52	46	8	43	50	10	52	40	Frontal Sup R	8
11	3.64	32	26	54	28	18	55	32	28	48	Frontal Sup R	8
53	4.31	−36	0	46	-35	-6	45	-36	2	42	Precentral L	6
13	3.72	−44	2	52	-42	-4	50	-44	4	48	Precentral L	6
19	3.66	−32	50	24	-31	43	29	-32	50	20	Frontal Mid L	9
11	3.37	18	−24	66	15	-30	61	18	-20	62	Precentral R	4
46	4.29	−16	−88	2	-16	-84	-2	-16	-85	6	Occipital Sup L	17
14	3.76	−30	−84	8	-29	-81	3	-30	-81	11	Occipital Mid L	18
12	3.64	26	−70	20	23	-69	16	26	-67	22	Occipital Mid R	18
11	4.24	−14	18	32	-14	12	34	-14	19	29	Ant. Cingulate L	32
10	3.62	−22	0	32	-22	-4	32	-22	1	29	Ant. Cingulate L	24
15	3.7	56	−36	6	51	-36	7	55	-35	7	Temporal Sup R	22
10	3.52	30	−36	−2	27	-35	0	30	-35	0	Hippocampus R	
12	3.56	22	−62	−48	20	-55	-44	22	-62	-37	Cerebellum R	
16	3.39	44	−72	40	39	-73	34	44	-68	40	Lobulus Parietal Inf	40